1: J Immunol 1997 Jun 1;158(11):5554-9

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Tullin S, Farris P, Petersen JS, Hornum L, Jackerott M, Markholst H.

Department of Immunogenetics, Hagedorn Research Institute, Gentofte, Denmark.

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PMID: 9164980 [PubMed - indexed for MEDLINE]

A Pronounced Thymic B Cell Deficiency in the Spontaneously Diabetic BB Rat[1]

Søren Tullin, Paul Farris, Jacob Steen Petersen, Lars Hornum, Malene Jackerott, and Helle Markholst[2]

In an attempt to elucidate the origin of the T cell lymphopenia and/or the β-cell-specific autoimmunity observed in diabetes-prone Bio-Breeding (DP-BB) rats, a thymic cDNA library was subjected to differential screening with thymic cDNA probes of DP-BB rats and nonlymphopenic nondiabetic controls. This approach resulted in the identification of a prominent lack of thymic B cells in DP-BB rats. This deficiency is distinct from a less pronounced peripheral B cell deficiency of different timing. The thymic B cell defect is linked to the lymphopenia trait on chromosome 4 and thereby with susceptibility to diabetes in crosses involving the DP-BB rat. In conclusion, our data suggest that the contribution of thymic B cells to the (negative) selection of thymocytes is inadequate in DP-BB rats, thus providing a plausible explanation for at least some of the spontaneous autoimmune phenomena in this animal model. ***The Journal of Immunology,*** **1997, 158: 5554–5559.**

The diabetes-prone Bio-Breeding (DP-BB)[3] rat spontaneously develops insulin-dependent diabetes (IDD or type 1 diabetes), with onset between days 60 and 120 (1, 2). In the DP line kept at the Hagedorn Research Institute the diabetes incidence has been >90% for generations (3). Regarding symptoms and pathogenesis, the diabetes of the DP-BB rat resembles the human disease. In both cases autoimmune destruction of the insulin-producing β-cells in the pancreatic islets of Langerhans is the major cause of the failing insulin production. The DP rats, however, display another unique phenotype; throughout life, the animals are severely T cell lymphopenic. The lymphopenia is characterized by a 4-fold reduction in the number of $CD4^+$ T cells and a 15-fold reduction in the number of $CD8^+$ T cells in peripheral circulation and lymphoid tissues (4–6). The absent T cells can also be defined by the expression of the RT6 surface molecule (7). Moreover, the remaining T lymphocytes of the DP-BB rat exhibit a reduced response to alloantigen in MLR (5–9) and to stimulation by mitogens (5, 10).

Genetic studies indicate that lymphopenia is caused by homozygosity for a recessive allele of a single gene (*Lyp* or *Idd1*), which has been mapped to the proximity of the neuropeptide Y gene on rat chromosome 4 (11, 12). IDD development, on the other hand, seems to be controlled by at least two genetic factors: one tightly linked to *Lyp*, and one linked to the MHC (MHC or RT1-complex in the rat) (13). As IDD is not observed in nonlymphopenic animals in crosses involving the DP-BB rat and a nonlymphopenic control, lymphopenia seems to be required for diabetes development in this animal model of human type 1 diabetes (3, 13–15).

The cellular defect(s) leading to T cell lymphopenia and diabetes has been analyzed in investigations in which bone marrow from DP-BB rats was transplanted into nonlymphopenic (nondiabetic) irradiated recipients and vice versa. These studies suggest that a deficient bone marrow causes most manifestations of DP-BB rat lymphopenia, including the reduced T cell number (16, 17), the absence of $RT6^+$ T cells (16), and predisposition to autoimmune type 1 diabetes (15, 16). Georgiou and Bellgrau characterized the cellular deficiencies even further; their results imply that predisposition to diabetes as well as the reduced response of BB rat T cells to alloantigen in MLR is determined by the origin of the thymic bone marrow derived APCs (9, 15, 16, 18). Somewhat in contrast to these findings, another study has shown that the reduced T cell number and the inability of the DP-BB rat to generate $RT6^+$ T cells is due to an intrinsic T cell deficiency (16). In conclusion, it seems plausible that two cellular defects play a role in the development of diabetes in the rat: one intrinsic to the T cell lineage, and another intrinsic to thymic bone marrow-derived APCs.

The aim of the present study was to identify genes with an aberrant expression in the DP-BB rat thymus by differential screening of a thymic cDNA library.

Materials and Methods

Animals

The DP-BB rats used in this study were from the HRI colony (3) kept at the Hagedorn Research Institute under specific pathogen-free conditions. Inbred NEDH (New England Deaconess Hospital) and BN (Brown Norway) rats were obtained from Møllegård Breeding Center (Lille Skensved, Denmark). In addition to the inbred animals, $(DP\text{-}BB \times BN)F^1 \times DP\text{-}BB$ backcross rats (19) were analyzed. All lymphopenic rats were screened for diabetes as outlined previously (19) from the age of 50 days. Thymi used for RNA isolation were obtained from 70- to 95-day-old rats. Serum samples were taken on day 35 or day 80. Whole blood for detection of T cell lymphopenia was taken before day 40. Thymi were removed at 30 to 40, 60, 80 to 90, and 120 days of age for flow cytometry and at 30 to 40 and 80 to 90 days of age for immunohistochemistry.

Department of Immunogenetics, Hagedorn Research Institute, Gentofte, Denmark

Received for publication February 13, 1996. Accepted for publication February 20, 1997.

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[1] This work was supported by the Danish Research Academy (fellowships for S.T. and L.H.) and the Juvenile Diabetes Foundation International (Career Development Award for H.M. and postdoctoral fellowship for J.S.P.). The Hagedorn Research Institute is an independent basic research component of Novo Nordisk a/s.

[2] Address correspondence and reprint requests to Dr. Helle Markholst, Hagedorn Research Institute, Niels Steensens Vej 6, DK-2820 Gentofte, Denmark. E-mail: hmar@hagedorn.dk

[3] Abbreviations used in this paper: DP-BB, diabetes-prone Bio-Breeding; IDD, insulin-dependent diabetes; NEDH, New England Diaconess Hospital; BN, Brown Norway.

0022-1767/97/$02.00

RNA isolation

Isolation of total cellular RNA was based on extraction with acidic guanidinium thiocyanate/phenol/chloroform (RNAzol B, Cinna/Biotex Laboratories, Inc., Houston, TX) as recommended by the manufacturer. Poly(A)$^+$ RNA was purified from total RNA on oligo(dT) cellulose columns using the mRNA purification kit from Pharmacia Biotech (Sollentuna, Sweden) as recommended by the manufacturer.

cDNA synthesis and cloning

cDNA was synthesized and cloned from the purified poly(A) RNA by using the Librarian II kit (Invitrogen Corp., San Diego, CA) essentially as recommended by the manufacturer. For construction of the thymic NEDH rat cDNA library, the eluted cDNA, with added linkers, was ligated into the pcDNAII vector and transformed into *Escherichia coli* as described in the Librarian II manual. The primary library consisted of 10^7 clones, with cDNA inserts above 500 bp in >80% of the clones and an average insert size of approximately 1300 bp.

Differential screening

The cDNA library from thymic cells of NEDH origin was screened as previously described (20). Briefly, plasmids from 50,000 colonies (spread on 35 petri dishes (13.7 cm in diameter) at a density of 1,000–1,500 colonies/dish) were transferred to and immobilized on 35 nylon membranes (Hybond-N membranes from Amersham, Aylesbury, U.K.). The membranes were probed sequentially with four thymic ^{32}P-labeled cDNA probes, two of DP-BB and two of NEDH origin, representing total cellular RNA of the entire thymus of individual rats. Between each hybridization the probe from the previous screening was removed from the nylon filters, followed by autoradiography of the stripped membranes to verify that all the probe had been removed. After hybridization and washing, the filters were placed on a paper towel that had been soaked in 0.5× SSC/0.2% SDS and wiped carefully on both sides with a second paper towel that had been soaked in the same solution. This treatment removed all background that remained after the traditional washing procedure.

Hybridization probes

Probes were labeled using Amersham's Multiprime DNA labeling system (based on random hexanucleotide-primed DNA synthesis) (21) and [α-^{32}P]dCTP (Amersham).

Northern blotting

Northern blotting experiments were performed as previously described (20). The PhosphorImager (Molecular Dynamics Ltd., Kemsing, U.K.) was used for analysis of the Northern blots. The PhosphorImager files were printed using linear gray scale adjustment, with white corresponding to a pixel value of zero (Figs. 2 and 3).

DNA sequencing

The cDNA inserts of plasmids, representing differentially expressed genes, were sequenced according to a slightly modified version of the Sanger sequencing protocol (22) using Pharmacia's AutoRead Sequencing Kit in combination with the A.L.F. Automatic Sequencer (from the same company) as recommended by the manufacturer. The kit-supplied, fluorescein-labeled universal primer was used as primer for the sequencing reactions.

Detection of lymphopenia

One hundred microliters of EDTA stabilized whole blood was incubated for 30 min at 4°C with 100 μl of PBS supplemented with 1% BSA (Sigma Chemical Co., St. Louis, MO; grade V), containing FITC-conjugated mouse anti-rat $\alpha\beta$ TCR-specific mAb (clone R73, Serotec, Oxford, U.K.). After washing, the erythrocytes were lysed by incubation at room temperature for 10 min with FACS lysing solution (Becton Dickinson, Ballerup, Denmark). Finally, after one additional wash the remaining cells were analyzed by flow cytometry. The fraction of TCR$^+$ cells among 10,000 leukocytes was determined using pre-established gatings on a FACScan (Becton Dickinson). An animal was considered lymphopenic if <20% of the leukocytes were TCR$^+$.

Immunohistochemical detection of thymic B cells

Rat thymi were suspended in Tissue-Tek (Miles, Inc., Elkhart, IN) on cork and snap-frozen in dry-ice-cooled isopentane. Using a Cryostat (Leitz 1720, Leica, Wetzlar, Germany), 5-μm serial sections of thymic tissue were collected and fixed by incubation in acetone for 10 min. For detection of total rat Ig, sections were preincubated with buffer containing normal rabbit IgG (Zymed, San Francisco, CA) and, after washes, incubated for 1 h at room temperature with biotinylated rabbit anti-rat IgG, IgA, and IgM (Serotec), diluted 1/50 in PBS supplemented with 0.25% BSA. For detection of the isoform of the leukocyte common Ag present on B lymphocytes only, sections were preincubated with buffer containing normal mouse IgG (Serotec) and, after washes, incubated for 1 h at room temperature with biotinylated mouse anti-rat CD45R/B220 IgG2b (clone His24, a gift from Dr. F. G. M. Kroese, Gronningen, The Netherlands) diluted 1/20 in PBS supplemented with 0.25% BSA. Following the washes, all sections were incubated with a streptavidin-peroxidase conjugate (Zymed). Finally, all sections were incubated with 3-amino-9-ethylcarbazole/chromagen solution substrate (Zymed) and counterstained with hematoxylin. The sections were examined by light microscopy (microscope type BH-2, Olympus, New Hyde Park, NY), and representative areas were photographed (Kodak 160 ASA film, Eastman Kodak, Rochester, NY).

Flow cytometric detection of thymic B cells

Single cell suspensions of thymocytes were obtained by gently teasing the tissue on stainless steel sieves in ice-cold PBS buffer supplemented with 1% BSA. Next, 10^6 cells were incubated for 1 h at 4°C in the presence of appropriate amounts of either two or three directly conjugated mAbs (FITC, red phycoerythrin, or biotin conjugates). The mAbs used were rat CD45-specific IgG1 (clone OX-1; PharMingen, San Diego, CA), rat CD45RA/B-specific IgG1 (clone OX-33; PharMingen), rat CD45RC-specific IgG1 (clone OX-22, PharMingen), and rat CD43-specific IgG1 (clone His17; PharMingen). OX-1 recognizes all isoforms of the leukocyte common Ag expressed on all hemopoietic cells except erythrocytes. OX-33 recognizes an isoform of CD45 present on B lymphocytes only (similar to B220-specific Abs). OX-22 reacts with high m.w. isoforms of CD45 found on B lymphocytes (and also on some peripheral T cells and NK cells) but only weakly expressed on thymocytes. His17 recognizes an epitope of Ly48/leukosialin that is expressed on all thymocytes but not on resting B cells. Following a wash and a 1-h incubation at 4°C with streptavidin-peridinin chlorophyll protein conjugate (Becton Dickinson) in the case of biotinylated primary Abs, the cells were washed and analyzed by flow cytometry. Routinely, the cell preparations contained >95% viable cells based on propidium iodide exclusion. One hundred thousand viable CD45-positive events were acquired using the FACScan (Becton Dickinson). The fractions of CD45RA/B$^+$ cells and CD45RC$^+$CD43$^-$ cells within the thymic cell suspensions were determined using the same four-dimensional attractor sets for the individual samples (Attractor software, Becton Dickinson).

Determination of rat serum Ig concentrations

Radial immunodiffusion assays specific for IgG1, IgG2a, IgG2b, IgA, and IgM (Serotec) were used for determination of the Ig concentrations in serum from DP-BB and BN rats (three animals 35 days of age and three animals 80 days of age in both groups) according to the manufacturer's instructions. In brief, serum samples were incubated at room temperature for 96 h, enabling the diffusion to run to completion, and the diameter of the precipitation rings to be measured. Standard curves for the individual Ig isotypes/subtypes were made by plotting the squares of the diameters of the precipitation rings obtained with the three kit-supplied Ig standards against their Ig concentrations in milligrams per milliliter. The standard curves were ultimately used for calculating the serum IgG1, IgG2a, IgG2b, IgA, and IgM Ig concentrations of the 12 tested animals.

Results

Identification of genes with aberrant expression in the DP-BB rat thymus

Fifty thousand colonies of a thymic NEDH rat cDNA library, spread on 35 plates, were screened sequentially with thymic cDNA probes from 80- to 90-day-old DP-BB or NEDH rats (two probes of each, four probes in total). Following visual inspection of the 35 sets of four autoradiographs, those clones that hybridized with only one of the probes were transferred to a new agar plate and rescreened. Autoradiographs of the rescreenings are shown in Figure 1, *A* and *B*, respectively. A comparison revealed approximately 30 clones giving rise to decreased/absent hybridization signals and approximately eight clones giving rise to an increased signal after probing with thymic cDNA from the DP-BB rat. The differential hybridization signals of the eight clones in the latter group, however, could not be confirmed when four additional thymic cDNA

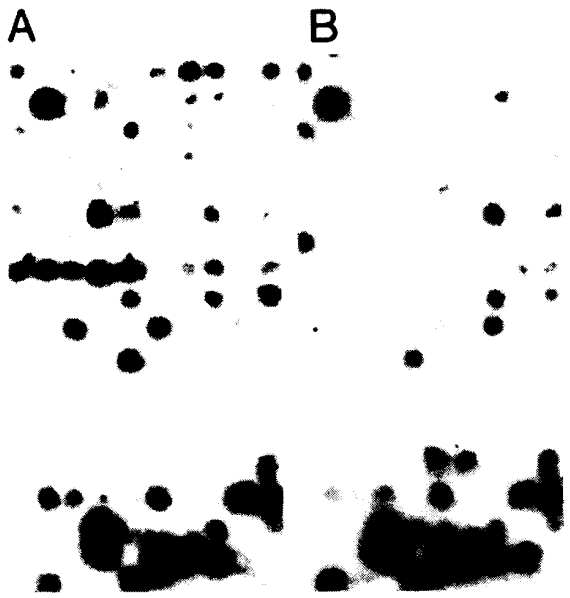

FIGURE 1. Screening of promising clones, isolated on the basis of the initial differential screening, with thymus cDNA of NEDH rat (*A*) and DP-BB rat (*B*) origin. The five clones represented by the dots in the lower part of the autoradiographs were included as controls, since they did not give rise to differential hybridization signals in the primary screening.

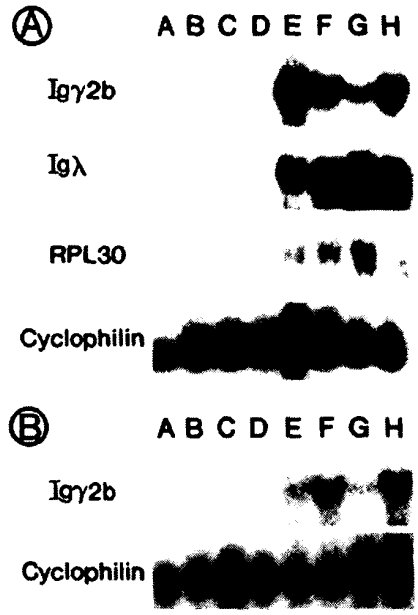

FIGURE 2. Northern blotting experiments with size-separated thymus RNA from 70- to 95-day-old rats. In *A*, RNA samples of four DP-BB rats (*lanes A–D*) and four BN rats (*lanes E–H*) were analyzed. The probes were Igγ2b cDNA, Igλ cDNA, Rpl30 cDNA, and cyclophilin cDNA, respectively. In *B*, RNA samples of four lymphopenic (DP-BB × BN)F1 × DP-BB backcross rats (*lanes A–D*) and four nonlymphopenic (DP-BB × BN)F1 × DP-BB backcross rats (*lanes E–H*) were analyzed. In this case the probes were Igγ2b cDNA and cyclophilin cDNA, respectively.

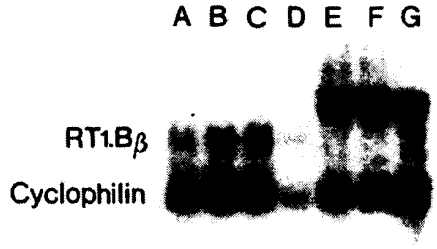

FIGURE 3. Northern blotting experiments with size-separated thymus RNA from 70- to 95-day-old rats. The RNA samples analyzed were from four DP-BB rats (*lanes A–D*) and three BN rats (*lanes E–G*). The blot was probed with a mixture of RT1.Bβ and cyclophilin cDNA.

probes were included in the screening. Consequently, these clones were not further investigated. By contrast, the decreased/absent hybridization signal of all 30 clones in the DP-BB rat was confirmed by all screenings.

Identity of the 30 differentially expressed cDNAs

Based on the restriction patterns of the plasmids from the 30 clones following digestion with various 4-bp restriction endonucleases, they were divided into 14 groups (data not shown). Partial sequencing of the cDNA inserts from one plasmid of each of the 14 groups revealed six cDNAs encoding the γ2b heavy chain component of the IgG2b Ig, five cDNAs encoding the λ Ig light chain, two cDNAs encoding the β-chain of the RT1.B MHC class II Ag, and one cDNA encoding a rat homologue of the human ribosomal protein Rpl30. Our NEDH rat RT1.Bβ DNA sequence showed 100% homology to the Lewis rat RT1.Bβ′ sequence (GenBank accession no. X56596) and 93% homology to the BB rat RT1.Bβ″ sequence (GenBank accession no. M24930). The homology between the rat Rpl30 DNA sequence obtained in this study and the human Rpl30 sequence (accession no. M94314) was 88%. The constant regions of our Igγ2b- and Igλ-encoding cDNAs showed 100% sequence homology to the constant regions of previously reported rat Igγ2b and Igλ cDNA sequences.

Igγ2b, Igλ, RT1.Bβ, and Rpl30 mRNA levels are reduced in the DP-BB rat thymus

The decreased expression in the DP-BB rat thymus of Igγ2b, Igλ, RT1.Bβ, and Rpl30 was investigated further in Northern blotting experiments with size-separated thymic RNA from lymphopenic and nonlymphopenic animals, all killed between days 70 and 95. In these experiments another nonlymphopenic inbred rat strain, the BN rat, was included to avoid detection of NEDH-specific peculiarities. Figure 2*A* shows pronounced differences in Igγ2b, Igλ, and Rpl30 hybridization signals between the DP-BB rat and the BN rat, i.e., the absence of Igγ2b, Igλ, and Rpl30 hybridization signals in lanes loaded with thymic DP-BB rat RNA and relatively intense hybridization signals in lanes loaded with thymic BN rat RNA. By contrast, the RT1.Bβ hybridization signal of the DP-BB rat RNA was only slightly reduced compared with the BN signal (Fig. 3). Interestingly, the DP-BB rat *u*-haplotype RT1.Bβ transcript (1.6 kb) was shorter than the BN rat *n*-haplotype RT1.Bβ transcript (2.0 kb).

Reduced Igγ2b and Igλ mRNA levels of the DP-BB rat thymus cosegregate with lymphopenia

To investigate whether the reduced Igγ2b, Igλ, RT1.Bβ, and Rpl30 mRNA levels segregated with lymphopenia in pedigrees derived from crossing the DP-BB rat with the BN rat, size-separated thymic total RNA of lymphopenic and nonlymphopenic (DP-BB × BN)F1 × DP-BB backcross animals (all killed between days 70–95) was probed with labeled Igγ2b, Igλ, RT1.Bβ, or

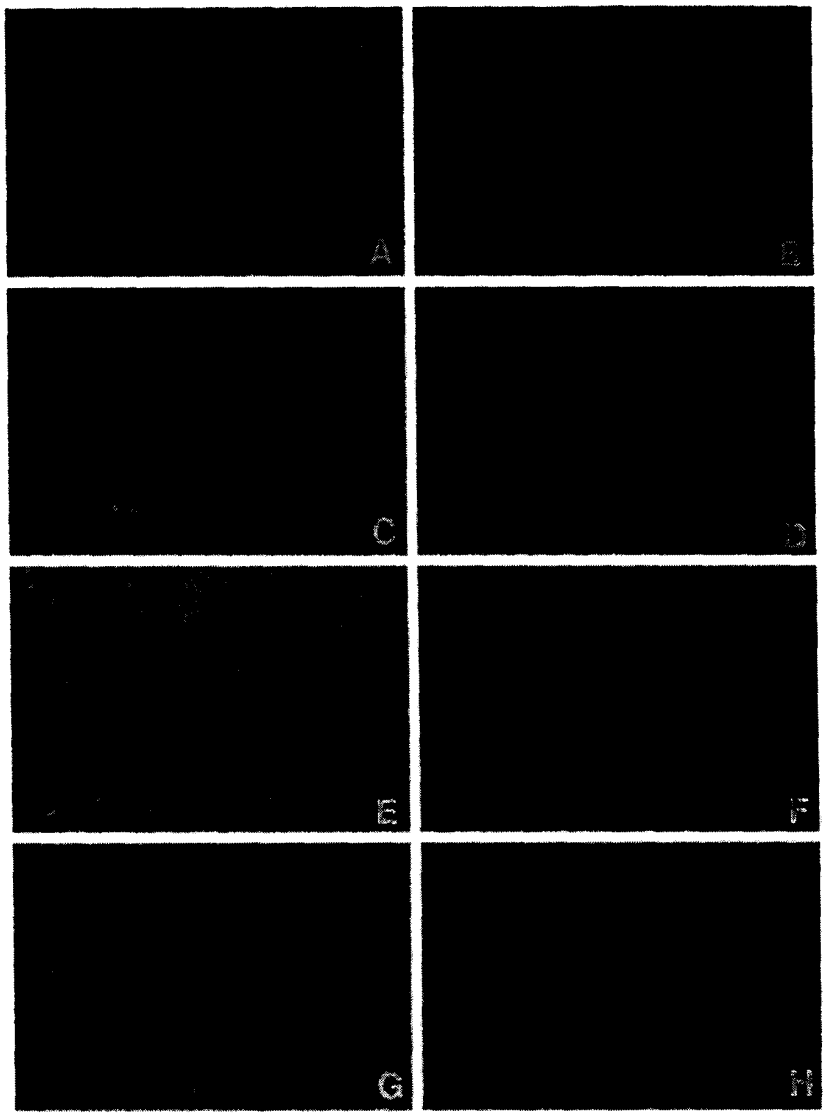

FIGURE 4. Immunohistochemical staining for B lymphocytes in thymic sections from 30- to 40- and 80- to 90-day-old rats (×20 magnification). The *four top panels* are representative slides stained with a polyclonal antiserum against rat Igs, while the *four bottom panels* are representative slides stained with the His24 mAb against the CD45R isoform of the leukocyte common Ag found on B cells only. *Top four panels*: *A*, a 35-day-old BN rat; *B*, a 35-day-old DP-BB rat; *C*, an 85-day-old BN rat; *D*, an 85-day-old DP-BB rat. *Bottom four panels*: *E*, a 35-day-old BN rat; *F*, a 35-day-old DP-BB rat; *G*, an 85-day-old BN rat; *H*, an 85-day-old DP-BB rat. In total, six BN and six DP-BB rats were analyzed at 30 to 40 and 80 to 90 days of age.

Rpl30 cDNA, respectively. All the lymphopenic animals investigated were diabetic. Figure 2*B* shows undetectable levels of Igγ2b transcript in thymic RNA samples from lymphopenic backcross rats and relatively intense hybridization signals in the lanes loaded with RNA from nonlymphopenic backcross rats. Perhaps as a consequence of long storage at −70°C of the thymus tissue from the eight backcross animals, the RNA isolated from these animals was partly degraded. This appeared not to be a problem when the RNA blots were probed for Igγ2b and cyclophilin (Fig. 2*B*). However, when the blots were probed for Rpl30, only a faint smear could be detected. Therefore, it is not possible at present to conclude whether the reduced Rpl30 transcript level observed in the inbred DP-BB rats cosegregate with lymphopenia in backcross offspring. Degradation of the RNA from the backcross rats also appeared to be a problem when the blots were probed for Igλ and RT1.Bβ. Nevertheless, the smeared results suggested that the lack of thymic Igλ message cosegregates with lymphopenia (data not shown). Moreover, the RT1.Bβ transcript size and hybridization signal appear to be linked to MHC haplotype, i.e., weak signal, short transcript to RT1^u and strong signal, long transcript to RT1^n (data not shown).

Total thymic Ig production is reduced in DP-BB rats

The deficient thymic Ig gene expression of the DP-BB rat was further characterized by immunohistochemical labeling of thymic cryostat sections with polyclonal Abs directed against total rat Ig. In the thymus of 30- to 40- and 80- to 90-day-old BN rats, clusters of positively stained, small, round cells were primarily located in the peripheral parts of the medulla, while only sporadic positive cells were noted in the cortex (Fig. 4, *A* and *C*). Such positively scored cells closely resembled positively stained cells of germinal centers of a lymph node (data not shown). In contrast to the BN rat, the thymi of 30- to 40- and 80- to 90-day-old DP-BB rats harbored considerably fewer positive cells, not located in clusters but as single cells in the peripheral part of the medulla (Fig. 4, *B* and *D*).

Number of thymic B cells is reduced in DP-BB rats

Thymic cryostat sections stained with the B lymphocyte-specific mAb, His24, showed markedly fewer positive cells in the corticomedullary junction and the medulla of 30- to 40- and 80- to 90-day-old DP-BB rats compared with age-matched BN rats (Fig. 4, *F–H*), confirming the data obtained on slides stained for total Ig.

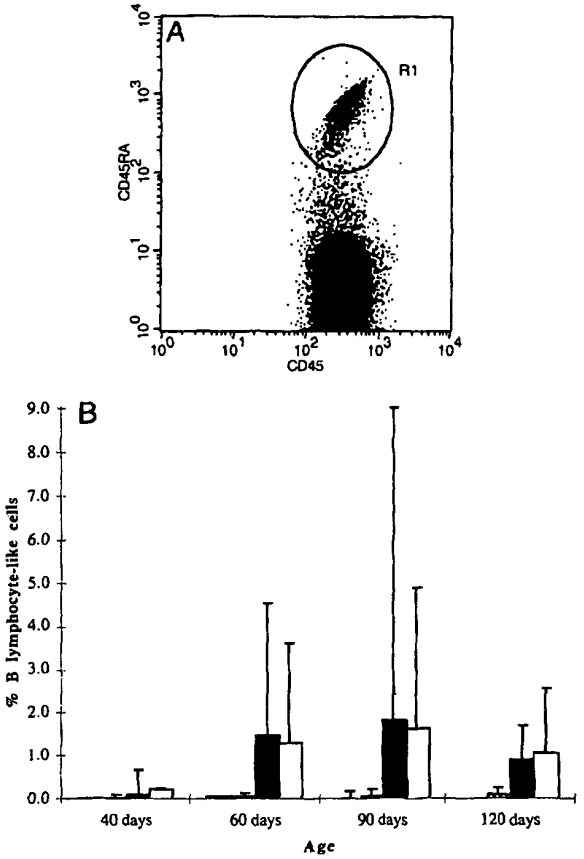

FIGURE 5. Fraction of B lymphocytes among thymocytes detected by immunofluorescence and flow cytometric analysis. *A*, Dot plot of 10^5 viable double-labeled thymocytes from normal BN rat shows a fraction of cells positive for the CD45RA/B surface molecule (present on B lymphocytes only) among all $CD45^+$ cells. *B*, Median fraction of B lymphocytes (with maximal range shown as error bars) at different ages. DP-BB rats (left- and right-hatched columns) were compared with BN rats (black and white columns). $CD43^-CD45RC^+$ cells among $CD45^+$ thymocytes are shown to the *left* (left-hatched and black columns), whereas $CD45RA/B^+$ cells among $CD45^+$ thymocytes are shown to the *right* (right-hatched and white columns). A total of 7, 5, 12, and 6 individual rats were compared with a total of 9, 6, 11, and 6 individual BN rats at 40, 60, 90, and 120 days of age, respectively.

Flow cytometric analyses of thymic cell suspensions immunolabeled with several mAbs specific for B cell surface Ags, including OX-33 recognizing the B lymphocyte-specific isoform of CD45 (Fig. 5*A*) showed that the fraction of thymic B cells increased from about 0.2% at 40 days of age to about 1.3% in 60-, 90-, and 120-day-old BN rats (Fig. 5*B*). Moreover, this analysis confirms that the fraction of thymic B cells was markedly reduced (>5-fold at 40 days of age to 10- to 20-fold at older ages; $p < 0.05$) within samples obtained from 40-, 60-, 90-, and 120-day-old DP-BB rats compared with those from age-matched BN rats, and that there was no increase with age (Fig. 5*B*). The fraction of cells identified as B lymphocytes with OX-33 corresponded to the fraction of cells identified as $CD43^-CD45RC^+$ (Fig. 5*B*).

Concentrations of IgG2a and IgG2b are low in serum from 35-day-old DP-BB rats, as opposed to the almost normal levels in 80-day-old rats

To investigate whether the Ig production from peripheral B lymphocytes in DP-BB rats was abnormal, the concentrations of IgG1, IgG2a, IgG2b, IgA, and IgM in serum samples from DP-BB and BN rats were measured in a radial immunodiffusion assay. The results showed serum concentrations of IgG1, IgG2a, IgG2b, and IgA in 80-day-old DP-BB rats to be comparable to the corresponding concentrations in 80-day-old BN rats (Fig. 6). In the young animals, however, the pattern was completely different: IgG2a (Fig. 6*B*) and IgG2b (Fig. 6*C*) were virtually absent from the serum of 35-day-old DP-BB rats; the concentrations were at least 50-fold lower than those observed with age-matched BN rats. In contrast, the concentration of IgA (Fig. 6*D*) in young DP-BB rats was roughly 4-fold higher than that in young BN rats. The IgM concentrations in both 35- and 80-day-old DP-BB rats were roughly 3 to 4 times higher than those observed in age-matched BN rats (Fig. 6*E*).

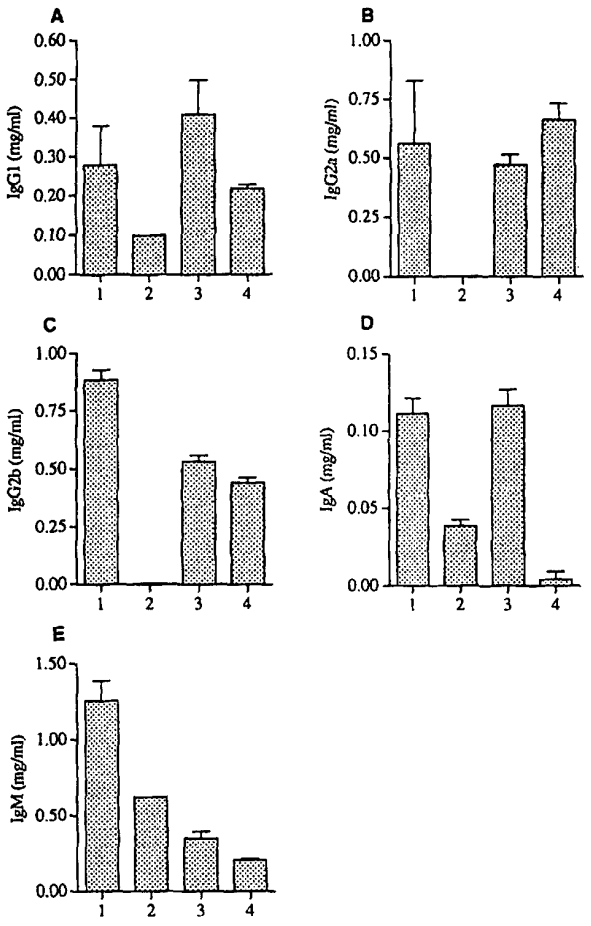

FIGURE 6. Concentrations in milligrams per milliliter of IgG1 (*A*), IgG2a (*B*), IgG2b (*C*), IgA (*D*), and IgM (*E*) in serum of 80-day-old DP-BB rats (*bar 1*), 35-day-old DP-BB rats (*bar 2*), 80-day-old BN rats (*bar 3*), and 35-day-old BN rats (*bar 4*).

Discussion

The present study describes a thymic B cell deficiency in the diabetes-prone BB rat. A thymic cDNA library was subjected to differential screening to identify genes with an aberrant expression in the DP-BB rat thymus. Four different groups of cDNAs were identified, all representing genes with a lower or absent expression in thymi of 70- to 95-day-old DP-BB rats. These encode the γ2b heavy chain component of IgG2b, λ Ig light chains, the β-chain of the RT1.B MHC class II complex, and a rat homologue of the human ribosomal protein Rpl30.

Immunohistochemical staining of thymic sections for total Ig indicates that the reduced expression of Igγ2b and Igλ in the DP-BB rat thymus reflects a reduction in the total thymic Ig production. Moreover, immunohistochemistry using B cell-specific mAbs as well as flow cytometry on thymic cell suspensions labeled for B cell-specific Ags demonstrates that the reduced expression of B cell-specific genes within the thymus of DP-BB rats is due to a prominent lack of thymic B cells.

A similar lack of B cells is not observed in the periphery (5). Furthermore, we observe a near-normal serum Ig repertoire in young adult rats. However, the isotype switching from IgM to IgG1, IgG2a, and IgG2b appears to delayed. Yet, insufficient T cell help due to the T cell lymphopenia could be a simple explanation for this delay.

The thymic B cell defect is not caused by the T cell lymphopenia, as our preliminary data show the nonlymphopenic DR-BB rat to share this phenotype. The thymic B cell defect and the lymphopenia are, thus, two independent traits, although seemingly genetically linked since no recombinants were observed among eight rats (Fig. 2*B*). The maximal distance between the two loci is calculated to be 37 cM (corresponding to the upper limit of the 95% confidence interval).

The importance of thymic B cells in negative thymic selection was demonstrated in a recent study in which purified macrophages, dendritic cells, or B lymphocytes from spleen or thymus of Mls-1a mice were injected into the thymus of neonatal Mls-1-incompatible animals. It was shown that only thymic B cells caused the depletion of Mls-1a-reactive Vβ6$^+$ T cells (23). Accordingly, we speculate that the thymic B cell deficiency in BB rats results in an inadequate negative selection of the maturing T cells, thereby contributing to the development of autoimmune diabetes and other autoimmune phenomena in this animal model.

The RT1.Bβ transcript size segregates with the MHC haplotype (i.e., short transcript to RT1^u and long transcript to RT1^n) in all backcross animals tested (data not shown), suggesting that transcript size is an inherent property of the two *u*- and *n*-RT1.Bβ alleles. However, it remains to be shown whether the transcript size difference has any biological significance, for example by predisposing homozygous RT1.Bβ^n/RT1.Bβ^u rats to autoimmune type 1 diabetes. The reduced hybridization signal observed for the RT1.Bβ^u transcript could be the consequence of the <100% (in this case 93%) sequence homology with the probe (the NEDH rat-derived RT1.Bβ^l nucleotide sequence). At present, it is difficult to say anything about the significance of the reduced level of the transcript encoding the rat homologue of the human ribosomal protein L30 (Rpl30) in the DP-BB rat thymus.

In conclusion, a new trait of the diabetic DP-BB rat has been described: a thymic B lymphocyte deficiency. This trait is genetically linked to but apparently different from the T cell lymphopenia also present in the DP-BB rat. Considering the importance of thymic B lymphocytes in the negative selection of thymocytes, we speculate that their absence can lead to an autoreactive pool of peripheral T cells. In concordance with this is the fact that the DR-BB rats (which are thymic B cell deficient but not T cell lymphopenic) can be rendered diabetic by depletion of regulatory T cells (24). This indicates that DR-BB rats also harbor autoreactive T cells. In our cross between DP-BB and BN rats only two loci segregated clearly with diabetes, namely the MHC (RT1) and the lymphopenia (Lyp) (19). However, the gene responsible for thymic B cell deficiency may be important for diabetes development in the rat, since the genetic contribution of this gene is masked by the linkage to Lyp.

Acknowledgments

We thank Bodil E. Andreasen, Susanne B. Rasmussen, and Ellen Ragnhof for technical assistance, as well as Troels M. Jørgensen and his staff for assistance with the maintenance of the BB rat colony. We also thank Dr. F. G. M. Kroese for his kind gift of the biotinylated His24 mAb, and Dr. O. D. Madsen for his valuable help with the immunohistochemistry.

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